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PINK DISEASE OF PLANTATION RUBBER.

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(With Plates XVI and XVII and 11 Text-figures.)

Introduction.

There has been a considerable development of Pink Disease in Malayan rubber estates since 1912 and at the present time it is the disease which requires the greatest amount of attention in many districts. We have seen several estates in which 10 per cent. of the trees were affected and in a few neglected plantations in parts of the country where the disease was bad no less than 25 per cent. were attacked. During 1914 an opportunity was given us of investigating this disease in detail especially as regards plantation rubber and the present paper embodies the chief results of this investigation. Other information about the disease, which is of interest only to planters, is included by us (4) in Bulletin 21 of the Department of Agriculture, Federated Malay States.

Distribution and Hosts.

Pink Disease is caused by a fungus which was named *Corticium javanicum* in 1901 by Zimmermann (17) who investigated it in Java with special reference to coffee, but Petch (9) points out that the same fungus had been named *Corticium salmonicolor* by Berkeley and Broome many years before from material obtained in Ceylon, hence the latter name has the right of priority.

In 1897 Ridley (11) reported the presence of a disease of coffee in Selangor caused by a fungus with pink spore masses, which was named

Necator decretus by Massee (8), and in 1901 Zimmermann (17) reported the same fungus on coffee in Java and pointed out that it was generally associated with *Corticium javanicum*, not only on coffee but also on tea and other hosts.

Between 1904 and 1909 the fungus was reported under different names on rubber in North Borneo (12), Malaya (6, 13, 14), Ceylon (9), and Southern India (1). More recently it has been found on rubber in Sumatra (10) and Burmah (4). *Corticium salmonicolor* has been found on other hosts in the Cameroons (5) and in the Caucasus (15). In the West Indies a pink fungus on cacao has been referred to *Corticium lilacofuscum*, which may possibly be identical with *Corticium salmonicolor*.

In 1912 Rant (10) published an account of an investigation of *Corticium salmonicolor* with special reference to cinchona, the most important result of which was to establish the identity of the *Corticium* and *Necator decretus*, these being shown to be two stages of one and the same fungus.

Corticium salmonicolor is an omnivorous fungus and Rant mentions that it has been found on no less than 141 species of plants belonging to 104 genera and many different families. The disease has been found on Gymnosperms as well as on Dicotyledons, but no record has yet been made of it on Monocotyledons. Rant (10) states he has seen the fungus growing on the epiphytic fern *Drymoglossum heterophyllum* without apparently causing harm.

In Malaya, *Corticium salmonicolor* has been found recently on Para rubber, cocoa, *Coffea robusta*, *Gardenia sp.*, *Hibiscus sp.*, camphor (*Cinnamomum camphora*), *Cassia sp.*, horse mango (*Mangifera foetida*), *Lansium domesticum*, lime, durian (*Durio zibethinus*), jak (*Artocarpus integrifolia*), *Averrhoa sp.*, mango (*Mangifera indica*), *Tephrosia Hookeriana*, *Indigofera arrecta*, and *Clithoria cajanifolia*. The number of fruit trees in this list is noteworthy, but the fungus is not often found on them.

In other tropical countries the fungus is of economic importance on other plants besides rubber. In Java, coffee and cinchona are seriously affected by it and in Ceylon the fungus causes a serious disease of tea.

Corticium salmonicolor is probably native in most of the countries in which it has been recorded. Many of the plants mentioned by Rant (10) as having been affected by it are native to Java and some of the plants on which the fungus has been found in Malaya are also indigenous. Anstead (1) states that the fungus is present on jungle

trees in the neighbourhood of rubber estates in Southern India. The fungus has probably spread from native hosts to plants that have been introduced, such as rubber, coffee, tea, and cinchona. There is one doubtful record of it on a jungle tree in Malaya, but one cannot say at present whether it occurs to any appreciable extent in the forests. Unfortunately *Corticium salmonicolor* has shown a considerable liking for rubber trees, and as far as Malaya is concerned *Hevea brasiliensis* is by far the commonest host for the disease. The disease is much more prevalent in some parts of the country than in others and there are some large areas where the disease has not yet been found. The disease is most abundant at present in the districts of heaviest rainfall and where large tracts of jungle remain.

Field Observations.

Pink Disease attacks rubber trees of various ages though it is not often seen on trees less than two years old. An attack often begins in a fork of a tree on account of the accumulation of water there, but sometimes the disease affects a branch in the middle and it has been seen occasionally to attack the main stem. The disease develops most rapidly during periods of heavy rain. In dry weather obvious signs of the fungus frequently disappear to appear again when the rains come.

The manifestations of Pink Disease on rubber trees are extremely variable. The disease is so called because the fungus often causes a pink incrustation on the branches or main stem, which is more specially developed on the under or shady side (shown on the right-hand side of Plate XVI, fig. 2). In this condition the disease is very striking and cannot be mistaken. The incrustation cracks irregularly after a time and the bright pink colour rapidly fades to a dingy white. There are, however, at least three other forms in which the fungus appears on rubber trees:

(*a*) Pink Disease frequently assumes the form of white or pale pink pustules arranged more or less in lines parallel with the branches. This is the "Höckerchen" form of Rant (10).

(*b*) At other times part of the fungus on the exterior consists of white or pale pink strands of a cobweb-like texture which run irregularly downwards over the surface, the strands being sometimes so delicate as to be overlooked (cf. Plate XVI, fig. 1). This is the "Spinnegewebe" form of Rant (10) and is usually the first stage to appear on an affected tree.

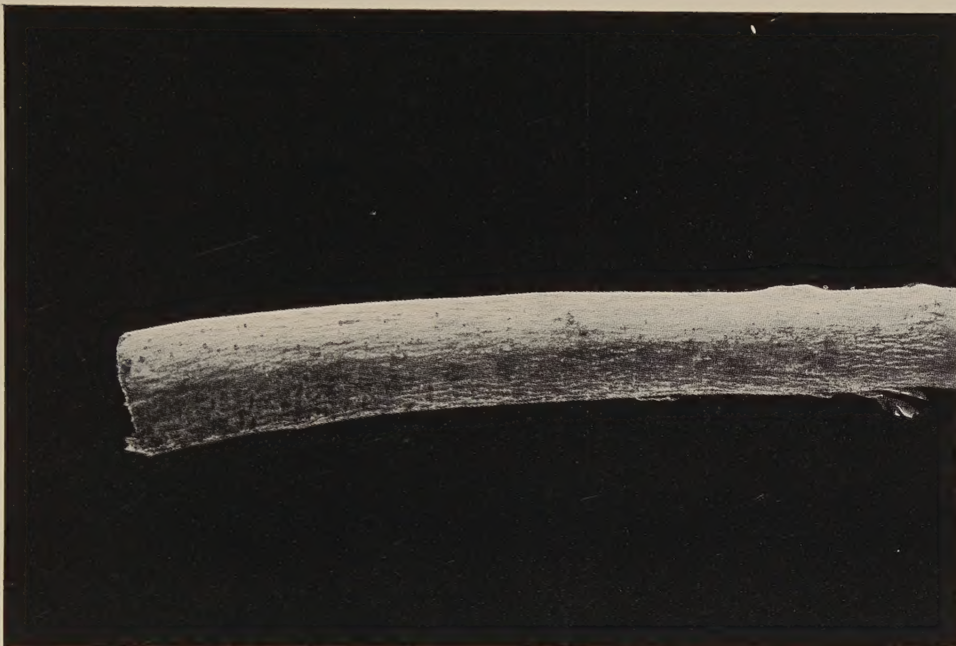


Fig. 2. Photograph of the incrusting form of *Corticium salmonicolor* (to right) and of pustules of the *Necator* stage (to left) on a branch of a rubber tree.

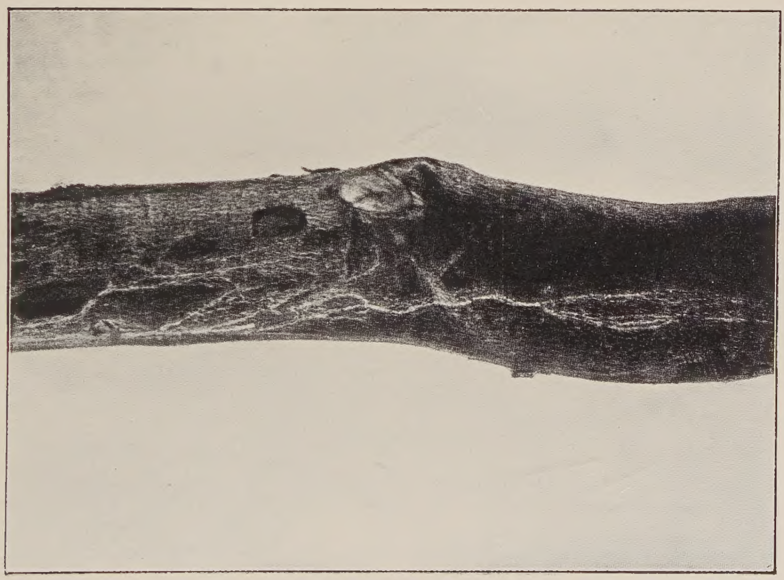


Fig. 1. Photograph of the cobweb-like form of mycelium of *Corticium salmonicolor* on a branch of a rubber tree.

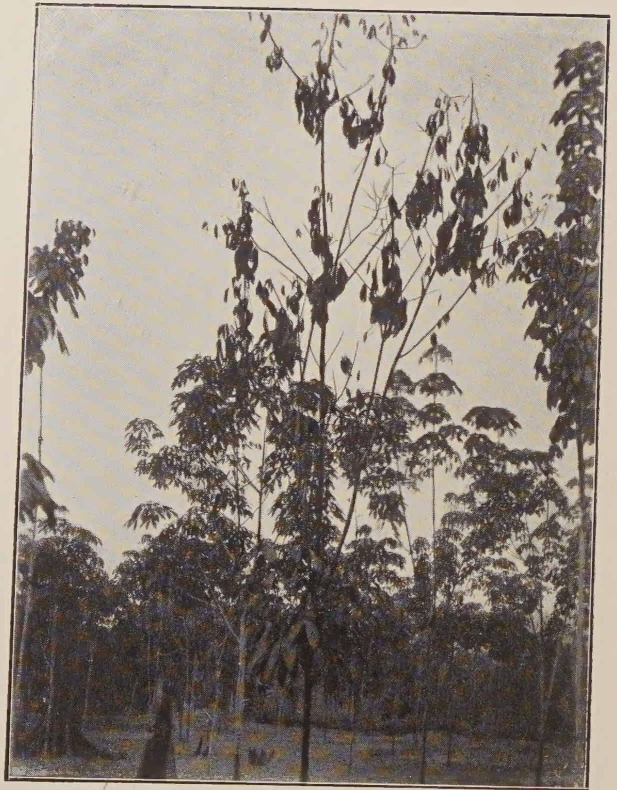


Fig. 3. Photograph of young rubber tree the upper part of which has been killed by *Corticium salmonicolor*.

Fig. 4. Photograph of rubber tree bearing "cankers" caused by *Corticium salmonicolor*.

(c) Finally there is the *Necator* stage which was formerly looked upon as a separate fungus, *Necator decretus*, Mass., but is now known to be a stage in the life-history of Pink Disease. Pustules of *Necator* are seen on the left-hand side of Plate XVI, fig. 2. The fungus in this condition consists of orange-red (not pink) pustules about one-eighth inch in diameter, each pustule being a mass of spores which serve to propagate the disease. In our experience the *Necator* stage has been confined to the side of the branch which is exposed to the brighter light. The *Necator* stage has always been found by us to be associated with other forms of Pink Disease, and so intimate is the connection between it and the other forms that it is difficult to understand the doubt that formerly existed as to the identity of *Necator decretus* and *Corticium salmonicolor*.

Spores of the fungus germinate on healthy bark especially where there is an accumulation of moisture. The mycelium which develops is entirely superficial at first, but after a time it penetrates the bark. When the mycelium reaches the laticiferous tissues, exudation of latex frequently begins and this runs down the bark and becomes blackened as time goes on. The weeping of latex from branches is often an indication of the presence of Pink Disease when from ground level no other sign of the disease can be seen. Once the mycelium has penetrated the bark it spreads upwards and downwards over and through the bark causing it to rot. The mycelium spreads more rapidly over the bark than through it. The fungus sometimes advances into the wood, this happening more frequently in small branches than in large ones. If the fungus spreads in the wood, the water supply becomes checked and the foliage of the affected branch turns brown and dies. On some undulating estates planters discover those trees which are affected by Pink Disease by observing from a hill the branches which are affected in this manner. Further information concerning the presence and development of the fungus in the wood is given below. Occasionally the fungus spreads downwards so vigorously that the whole of the upper part of the tree dies. In such a case as that represented in Plate XVII, fig. 3, the lower part of the tree sometimes makes an effort to recover by putting out new branches. When large branches are attacked by *Corticium salmonicolor* the progress of the fungus in the bark may be checked by a spell of dry weather and in this case an open, canker-like wound such as is shown in Plate XVII, fig. 4 is often formed as described by Petch (9). The formation of a callus on the margin of the wound tends to repair the injury and occasionally the disease is entirely

thrown off, but the fungus sometimes develops again over the bark which began to close the wound. Where the cankered areas have entirely thrown off the disease, the region around them is frequently blackened on account of oxidation of the rubber exuded when the disease was active.

Investigation of Hevea wood affected by Corticium salmonicolor.

Before the appearance of Rant's paper (10), Petch (9) stated that *Corticium salmonicolor* does not enter the wood to any appreciable extent. Rant noticed in cinchona that the fungus invaded the wood and the pith.

Branches of *Hevea brasiliensis* attacked by Pink Disease die in a manner characteristic of those attacked by a fungus which grows vigorously in the wood. Young branches are more often affected in this manner than are old ones. The rapid death of the leaves in such branches is a sure sign that the water supply is checked: this restriction is probably due to the activities of the fungus in the wood. It soon became clear that the fungus entered the wood of such branches, so a detailed investigation of the manner in which it invaded these tissues was made. Wood affected by *Corticium salmonicolor* is only slightly discoloured and differs greatly in this respect from wood permeated by *Diplodia cacaoicola*.

During the early part of 1914 a large branch of a rubber tree attacked by Pink Disease was obtained from an estate in Negri Sembilan in which the transition between healthy and diseased wood could be clearly traced. The branch was covered with the pink incrustation which ran out below into the cobweb-like form of the fungus. The cortical layers were dead and could be easily stripped off exposing the wood beneath.

When split longitudinally, the wood was seen to be sound for about two feet from the top of the branch, but the part below was dry and obviously diseased, except for a length of about nine inches which marked the transition between the healthy and diseased wood. This transition region had a moist, almost transparent, appearance and gradually passed below into the dry, diseased wood and above into the moist, healthy wood.

Diplodia cacaoicola, P. Hennings, is a common wound parasite of *Hevea brasiliensis* and has been found in some branches attacked by Pink Disease. A careful search for *Diplodia cacaoicola* was therefore made before investigating the mode of attack of *Corticium salmonicolor*. No external sign of *Diplodia* was observed and microscopical examination

failed to show any of the characteristic dark coloured hyphae of this fungus running through the vessels even in the most badly attacked portions of the wood.

Though *Diplodia cacaoicola* was absent, the wood was permeated with hyaline hyphae. A section of the branch including the transition area and a portion of the dead wood was taken and cut into numbered blocks throughout its length. Razor sections of the wood at different levels were made, the lower portion of the transition area being found most favourable for examination. Transverse sections through this part show the hyphae ramifying through the elements of the wood, being especially prominent in the vessels (Fig. 1). The wood of *Hevea*

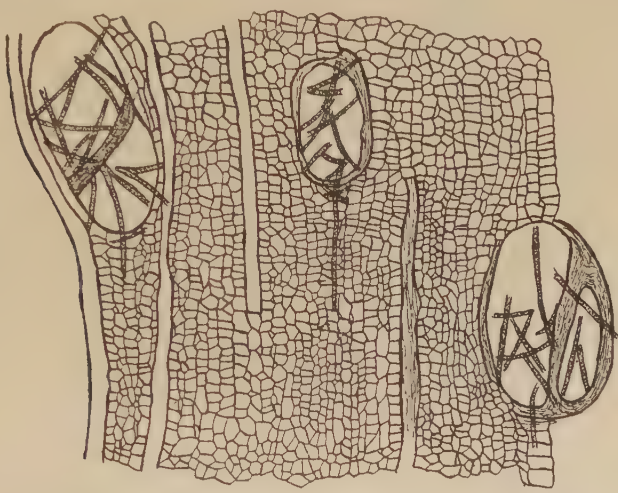


Fig. 1. Transverse section of wood showing hyphae in the vessels. ×40.

brasiliensis is mostly composed of fibrous elements together with a comparatively small number of large vessels and narrow medullary rays.

A study of longitudinal sections, both radial and tangential, shows the nature of the attack upon the wood. A favourable radial section shows the hyphae passing transversely through the wood along the medullary rays by way of which also the fungus passes from the bark into the wood. The fungus obtains food from materials stored in the ray cells which become filled with septate hyphae (Fig. 2). In radial section the medullary rays appear to be broad bands of infected tissue passing through the wood. At places where the vessels meet the

medullary rays, the mycelium travelling in the cells of the rays spreads out and enters the vessels (Fig. 3). All the elements of the wood become permeated with the hyphae which pass readily through the large pits without constriction. The deep medullary rays favour a quick passage transversely while the large pits allow a ready passage for the hyphae among the elements of the wood.

The most characteristic feature in the wood of *Hevea brasiliensis* attacked by Pink Disease is the presence of tyloses in the vessels (Figs. 4 and 5). Every specimen examined showed these bladder-like ingrowths from the living cells bordering the vessels, plugging up

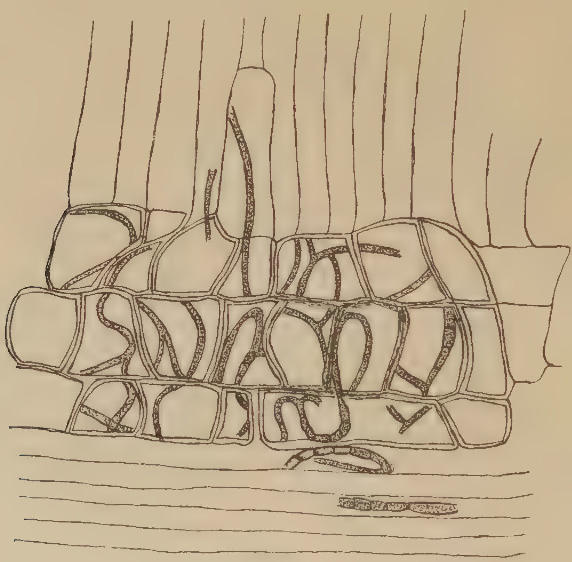


Fig. 2. Section showing hyphae in medullary ray cells. ×160.

the water courses. Specimens of healthy wood, of wood taken below the tapping area, and of wood attacked by *Diplodia cacaoicola* were examined, but in no case was there any indication of tyloses. Thus the formation of tyloses in *Hevea brasiliensis* appears to be a response to the attacks of *Corticium salmonicolor*.

The tyloses are of two types, (*a*) those in which the cells retain their thin cellulose walls (Fig. 4), (*b*) those in which the walls become lignified (Fig. 5). Both types are found in the same branch in adjoining vessels though the second type is rarely produced. The tyloses which become lignified lose their protoplasm and appear like

a number of small vessels included in a larger one. In the majority of cases only the thin-walled type occurs. Their contents are devoid of food reserves.

The response made by living cells to an injury usually results in an abnormal growth of neighbouring cells. In *Hevea brasiliensis* attacked by Pink Disease an abnormal bladder-like ingrowth of the

Fig. 3. Longitudinal section showing hyphae passing from medullary rays into vessels. Hyphae pass through without constriction. (Drawing somewhat diagrammatic.) × 160.

living cells bordering the longitudinal path of the fungus, *i.e.* the vessels, takes place.

The formation of tyloses as a traumatic response is presumably an attempt on the part of the host to check the passage of the fungus through the tissues. In this case it is obviously unsuccessful as the hyphae easily pass through the tyloses (Fig. 4). Failing this function,

however, they hasten the death of the branch by preventing the ascent of water.

This investigation of diseased branches explains the symptoms often observed when branches of *Hevea brasiliensis* are affected by Pink Disease. The fungus attacks the wood, vigorously pursuing its course up and down the stem through the vessels. Other vessels are blocked with tyloses for considerable distances while others again are filled with a brown gummy substance. Thus a large proportion of the functional

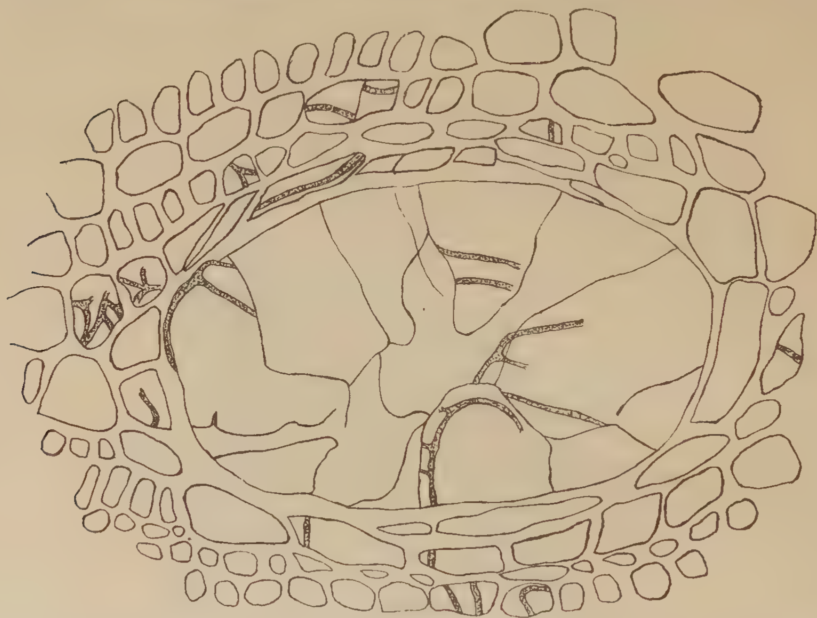


Fig. 4. Transverse section of vessel with thin-walled tyloses, showing hyphae passing through tylose cells. ×160.

vessels are rendered useless. This results in a serious diminution in the amount of water ascending to the leaves which droop and ultimately die.

Description of the Fructifications of the Fungus.

(*a*) *Basidial stage.* Zimmermann is responsible for placing the fungus in the genus *Corticium* and one gathers from his description and the accounts of subsequent writers that the pink incrustation is the basidial fructification. We found, however, that this stage was sterile in more than 80 per cent. of the large number of cases examined at

Fig. 5. Transverse section showing vessel with lignified tyloses. $\times 160$.

Fig. 6. Section of pink incrustation with basidia. Section 6μ thick and stained with Coton Bleu and Orange G. $\times 400$.

different times of the year. Great difficulty was experienced in finding basidia at all and it was not until after much searching that we recognised the type of incrustation that produced basidiospores. This form is thicker, has a more homogeneous surface, and when dry cracks into larger pieces than the sterile incrustation. It is remarkable that neither Zimmermann nor any other writer has called attention to the fact that the pink incrustation is frequently sterile.

One concludes from Zimmermann's description and figures that a typical hymenium is developed, but according to our experience the basidia are scattered and are irregularly arranged as in Fig. 6. The size of the spores is as given by Zimmermann and the sterigmata are noticeably long. We have not seen the basidia arranged even approximately as they are in Zimmermann's figure. The irregular distribution of basidia reminds one rather of an *Hypochnus* than a *Corticium.* Rant (10) has apparently made no special study of the basidial stage as he copies Zimmermann's figure of the hymenium and agrees with his description of it.

In North America, Stevens and Hall (16) have described a disease of pomaceous fruit trees caused by *Hypochnus ochroleucus*, Noack, which spreads over branches and twigs by means of mycelial strands and kills the leaves by enveloping them. The basidia are scattered and are irregular in form. *Corticium salmonicolor* seems to be more closely related to *Hypochnus ochroleucus* than to other species of *Corticium.* Bernard (3) also has described a disease of tea in Java which he attributes to a fungus named by him *Hypochnus theae.* Though there appear to be certain minor differences between this fungus and *Corticium salmonicolor,* the resemblances in the arrangement of the basidia and the character of the sterigmata and spores are very striking.

(*b*) *Necator stage.* The genus *Necator* was founded by Massee (8) in 1897 for the reception of a single species, *Necator decretus*, which was the cause of a stem disease of coffee in Malaya. It is now known that this is one of the stages of *Corticium salmonicolor.* The *Necator* stage consists of orange-red masses of spores, the individual spores being irregular in shape (Fig. 7) and hyaline when seen under the microscope. Each spore mass is waxy in consistency and it is likely that the spores become separated from one another only in wet weather when they are washed apart.

The mode of formation of these pustules is different from what one would gather by examining Zimmermann's figures, which have also been reproduced in Rant's paper (10). Zimmermann's figures

indicate an origin somewhat similar to that of a pycnidium, but we find that the mycelium aggregates beneath the outermost layer of cells of the branch, forming a kind of stroma which by growth ruptures the

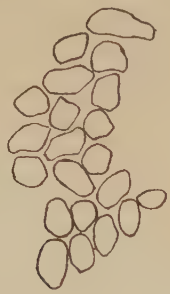
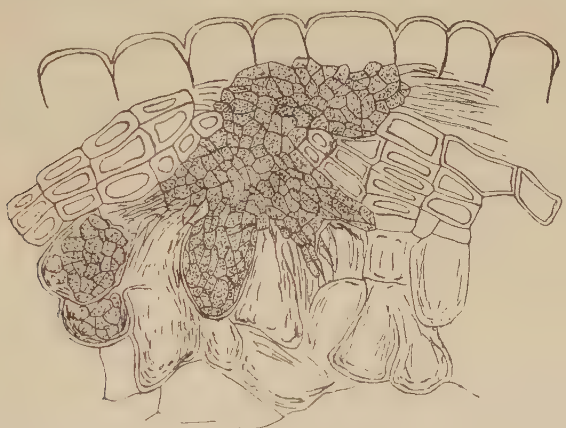


Fig. 7. Group of *Necator* spores, teased out, showing irregular size of spores. ×400.

tissues of the host (Figs. 8, 9). The whole of this stromatic mass becomes converted into spores by the separation of the cells one from the other. The irregularity in the size and shape of the spores (Fig. 7) is due to this peculiar method of spore formation. The dimensions of the



Figs. 8. Section showing early stage in development of a *Necator* pustule: Stroma forming under the outer layer of cells. ×160.

spores are 14–20 $\mu \times$ 8–10 μ. In other species of *Corticium* and *Hypochnus* small sclerotia about the size of pustules of *Necator* are produced and in *Corticium salmonicolor* such sclerotial aggregates may have become modified into spore masses by separation of the constituent cells instead of forming resting bodies.

Necator spores germinate readily in distilled water and in nutritive solutions (Fig. 10).

Fig. 9. Section of fully developed *Necator* pustule. ×400.

In Malaya the *Necator* has been found much more frequently than the basidial stage and it is likely that it takes the more active part in the dissemination of the disease.

The other forms of *Corticium salmonicolor* are constantly sterile.

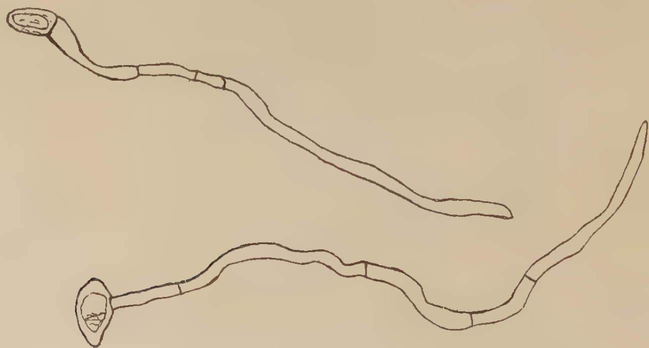


Fig. 10. Germination of *Necator* spores, after 12 hours in damp chamber. ×400.

The fungus is probably chiefly spread by wind, though it is possible that it is also disseminated by red ants and other insects which visit rubber trees. It is possible too that not only spores but also small portions of the sterile incrustation are disseminated by these means.

The incrustation retains its vitality for a considerable time and, as stated above, it cracks into small pieces as it gets older, and these, breaking away, may be carried to other trees in one or other of the ways mentioned.

A species of *Nectria* is often associated with cases of Pink Disease of long standing, but as far as is known at present it is purely saprophytic and develops only after the bark has been killed by *Corticium*.

Pure Cultures of Corticium salmonicolor.

At the beginning of these investigations efforts to obtain pure cultures of the fungus were unsuccessful. We soon recognised that the incrusting form was usually sterile so we gave up attempts to obtain a deposit of basidiospores with which to start pure cultures. Failing basidiospores, small pieces of the pink incrustation were cut out with a sterile knife and placed on slants of salep agar. The fungus quickly developed, but the cultures were usually contaminated. Subcultures started from these were eventually obtained which were probably pure. No further attempt to obtain pure cultures in this way was made because at this stage one of us obtained material in the field which appeared somewhat unusual at the time. This material bearing orange-red pustules was sent to the laboratory and immediately examined, when the pustules were seen to consist of masses of spores, these being the *Necator* form of the fungus which was subsequently obtained frequently.

Pure cultures were obtained from the *Necator* spores. These were teased out on sterile glass slides; some were placed in damp chambers on salep agar, others directly on test tube slants of the same medium. The spores quickly germinated in the damp chambers (Fig. 10); these were kept under observation for three or four days in order to see whether the cultures remained pure. After this, transfers from the damp chambers to test tube slants were made. As these cultures and those obtained by placing spores on test tube slants direct were identical, little doubt remained as to their purity. The mycelium was pure white and did not grow copiously. After a period of about ten days a faint pink colouration appeared in the cultures. The agar cultures were kept for several weeks, but no further development took place. Other cultures were then made by transferring small portions of the mycelium to blocks of sterilised *Hevea* wood placed in tubes (a figure and explanation of the form of tube used is given in Fig. 11). About 50 per cent.

of the attempts to start cultures from small pieces of mycelium were unsuccessful.

The cultures on wood blocks obtained in the first place from *Necator* spores develop quickly. The mycelium grows profusely and remains white for seven to ten days. It spreads over the block and into the

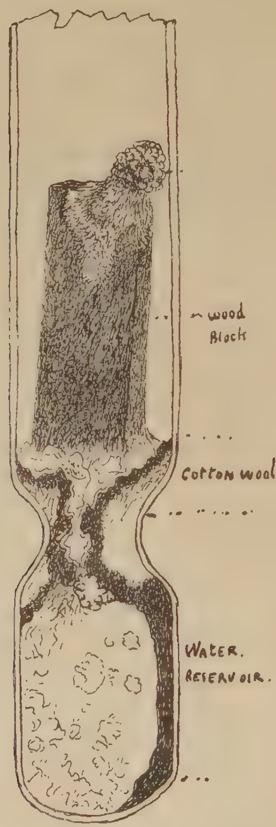


Fig. 11. Culture tube: wood block covered with mycelium which has grown into water reservoir covering the water with a film of hyphae on which *Necator*-like masses have formed. Large *Necator*-like masses at top of wood block.

cotton wool placed at the base of the tube. Growing in the cotton wool the mycelium begins to turn a pale pink colour which gradually spreads over the whole culture. Subsequently the mycelium in the cotton wool becomes a bright rose colour. The mycelium passes from

the cotton wool into the reservoir of water where it becomes aggregated.

The cultures started originally from the pink incrustation were now used to inoculate sterilised blocks of *Hevea* wood. The cultures so obtained were identical with those derived from the *Necator* spores so that, apart from other evidence, this alone indicated the connection between the two forms.

The cultures on wood blocks were placed under different conditions to ascertain whether these influenced the development of the colouration. Some were placed in the laboratory in diffuse light, others in direct sunlight, and others in darkness. Rant (10) states that his agar cultures placed in darkness remained white though after illumination for a short time a pink colouration appeared. In our experiments the cultures grown in darkness behaved like those in the light except that the pink colour did not develop so rapidly.

In older cultures the mycelium often turns a dirty brown colour as though some impurity had entered. However, small portions of this mycelium seen by transmitted light show the characteristic colouration. The hyphae in the old brown cultures are closely aggregated, corrugated, septate, much vacuolate, with numerous clamp connections. The hyphae in young cultures are septate, vacuolate, but not corrugated, whilst clamp connections are less numerous.

In one culture placed in bright light and in another kept in diffuse light an aggregation of hyphae took place along the upper edge of the blocks to form a bright pink mass. The aggregation continued until a solid mass half an inch high and half an inch in diameter was formed, attached to the block by a thinner base. It resembled a number of closely attached *Necator* pustules (Fig. 11). Examined microscopically the mass was found to consist of short cells somewhat irregular in size forming a kind of pseudo-stroma.

These *Necator*-like masses are often formed in the cotton wool at the base of the culture tubes and sometimes upon the surface film of mycelium in the reservoir. Their spore-like nature is indicated when small pieces are used to start new cultures upon wood blocks. In every case the mycelium develops copiously within 24 hours, while, as stated above, 50 per cent. of attempts to start new cultures with portions of the usual form of mycelium result in failure. Under natural conditions it is probable that the cells forming these pustular masses would become detached from one another in a manner similar to those forming typical *Necator* pustules.

Rant (10) states that he did not obtain either fruit form in his cultures, but calls attention to the formation of "paraplectenchymatische mycelienknäuel," which he obtained now and again in his cultures and which had been previously observed by Koorders (7) in cultures of *Necator*. The *Necator*-like masses obtained by us probably correspond to these structures observed by Rant and Koorders. Rant confined his attention chiefly to agar cultures, but in our cultures on this medium we did not obtain anything approaching a fruiting form, though our efforts in this direction were not long continued after we found wood blocks so favourable for culture work.

Some of the wood blocks on which cultures had been grown were sectioned. It was seen that the fungus had spread to the centre of the block and that the mycelium had penetrated the elements of the wood in the same manner as in wood naturally infected. Tyloses were absent.

Inoculation experiments.

Rant's inoculation experiments (10) experimentally demonstrated the connection between *Corticium salmonicolor* and *Necator decretus*. Our inoculation experiments to be now described were carried out to test the conditions which favour or hinder the development of the fungus on *Hevea brasiliensis*. Correlated experiments upon other hosts were kept under observation at the same time.

The first series of inoculation experiments was carried out on rubber trees three-and-a-half years of age. The inoculations were made on the 4th of February, 1914, with pieces of the pink incrustation obtained from a diseased rubber tree. The inoculations were covered with cotton wool pads which were moistened every morning for the first three weeks as the weather was dry during this period. The pads were kept in place by rubber bands.

Little rain fell between the date of inoculation and March 10th, but between March 10th and March 16th there were daily showers. The fungus appeared during this wet spell, 13 out of 29 inoculations being successful. Of this number 8 out of 13 (60 per cent.) were obtained upon uninjured parts; 5 out of 16 (30 per cent.) upon wounded surfaces. These results provide further evidence that *Corticium salmonicolor* acts rather as a vigorous parasite on uninjured parts than as a wound parasite.

Between March 16th and April 14th the weather was dry and no rain fell for considerable periods. On the latter date only a few trees

showed any trace of the fungus and it appeared that the fungus was dying or was hibernating in the bark.

The inoculations were examined from time to time, but the fungus made no further progress. This result may be attributed to the dry weather which followed the moist spell ending on March 16th. A similar result is often observed on estates, for branches of rubber trees may recover from an attack of Pink Disease if a long spell of dry weather intervenes.

Similar experiments were carried out in the field at the same time on *Coffea liberica*, *Cinchona succirubra*, *Cinchona ledgeriana*, and *Cinnamomum camphora*, six inoculations being made in each host, three being in wounds and three being on unwounded surfaces. Only one successful inoculation was obtained and that on *Coffea liberica* where the cobweb-like form of mycelium appeared over the wound in which the fungus had been inserted.

The results indicate that these hosts are attacked less vigorously than is *Hevea brasiliensis*. These experiments indicate the possibility that small portions of the sterile incrustation may disseminate the disease. These easily break away and are blown about by the wind. Under favourable conditions the mycelium may develop and give rise to a new infection.

Inoculations were subsequently commenced in the laboratory with pure cultures of the fungus. Rubber seedlings and plants of *Gardenia sp.* and *Cinchona succirubra* were inoculated either by tying wood blocks used in growing pure cultures upon the stem, or by placing pieces of the mixture of mycelium and cotton wool from the base of tubes in wounds in the stem or in contact with the stem. Six inoculations of rubber seedlings and of *Cinchona* and three inoculations of *Gardenia* were made on July 10th, but by August 19th no success had been obtained. In these experiments the plants were kept under bell jars. The inoculations on *Gardenia* and *Cinchona* were overgrown with moulds within a week. In several inoculated rubber seedlings, however, the fungus appeared to grow strongly at first, but it soon weakened, and on August 19th the host plants were quite healthy. The experiments indicate that Pink Disease is not likely to attack very young plants of Para rubber.

Inoculations of rubber trees with pure cultures were also carried out in the field, trees standing in an overgrown nursery being used for this purpose. Fairly large branches of the outside trees were inoculated on July 14th with the mixture of mycelium and cotton wool from the

base of the culture tubes. Similar experiments were performed at the same time with *Coffea liberica* as host. The following table summarises the results on August 19th:

Name of host	Type of inoculation	No. of inoculations	No. of successful inoculations
Hevea brasiliensis	placed in contact	12	1 cobweb-like form
	placed in wounds	12	1 cobweb-like form
Coffea liberica	placed in contact	4	1 cobweb-like form
	placed in wounds	4	——

Thus successful inoculations of both rubber and coffee were obtained with pure culture material of the fungus.

Rant experimentally demonstrated that *Corticium salmonicolor* does not exhibit the phenomenon of specialised parasitism. Thus this fungus occurring on one host is not limited in infective power to that particular host or a few others, but can attack a wide circle of plants. It passes readily under favourable conditions from one host to another. Only in very wet weather does the fungus spread rapidly, as was obvious in our field experiments. Relatively few of our inoculations were successful and this we attribute to the difficulty of keeping them moist during the spells of dry weather which intervened during the progress of the experiments.

Treatment.

It must be pointed out in the first place that, exceptional cases apart, spraying with fungicides is impracticable and to some extent also useless. Spraying a rubber plantation with trees 30 to 60 feet high is an entirely different proposition from spraying an orchard containing trees only 20 feet or so high. There would have to be something in the nature of a revolution in spraying methods to enable a mature rubber plantation to be sprayed effectively so as to check Pink Disease. Spraying experiments which we recently carried out with two modern machines provided with extension rods show that the maximum height for which they can be used under estate conditions is 25 to 30 feet, and this height is only obtained with difficulty by coolie labour. Longer extension rods have been tried, but the difficulties of manipulation under estate conditions are so great that they cannot be used with

success for spraying large numbers of trees. Hence if a mature rubber plantation were sprayed for Pink Disease the upper branches of the trees would remain unprotected and these are the parts most liable to the disease. Again, in view of the regularity of the rainfall in the Federated Malay States a single spraying would be useless. To be effective at all in such a climate, spraying would have to be repeated at frequent intervals.

Spraying with Bordeaux Mixture or Lime Sulphur, preferably the former, might be effective in checking the disease in plantations not more than three years of age if there was danger of it breaking out in epidemic form, but fortunately there is yet no indication of this in Malaya. In Southern India where there is a prolonged dry season, Anstead (2) reports that painting the forks of young trees with Bordeaux Mixture before the coming of the monsoon reduced the percentage of trees affected from 1·34 to ·56, ·07 (three applications of the fungicide were given here), and ·7 per cent. in various cases. In our opinion estates infected to the extent of about one per cent. would be preferably treated by cutting out or by tarring as described below. Another circumstance, which would only occur exceptionally, and in which spraying a limited number of trees might be undertaken, is where the disease is confined to one portion of an estate. In conjunction with treatment within the infected area in the manner described below, it might be advisable to spray carefully a belt of trees around this area as a precautionary measure.

When Pink Disease first appears in a rubber plantation it is usually distributed in a sporadic manner. It is of the utmost importance that the disease should be dealt with vigorously from the outset by cutting off and burning the affected parts. In most plantations where Pink Disease appears for the first time only a few trees are attacked. In such cases diseased branches should be cut off at least two feet below the lowest point where there are obvious signs of the fungus and it is preferable to cut them off flush with the main stem or larger branch.

Where a large number of trees are affected on an estate the manager will probably hesitate before he cuts out the disease in this drastic manner. As an alternative, branches and main stems which appear to have a chance of recovery should be covered with tar for two feet above and below the region over which the fungus is evident. If the disease is dealt with in this way in the early stages many branches and sometimes entire trees may be saved. Even when the fungus has penetrated the bark to a slight extent the external application of tar

appears to check its progress. It has been urged that the diseased bark should be removed before tar is applied or even that tar should first be placed over the affected parts, the rotten bark removed, and then tar subsequently applied again. These are excellent ideals and if expense were no object would be strongly recommended. Experience has shown, however, that where tar is applied thoroughly without previous removal of diseased bark good results are obtained as long as the treatment is renewed within a month if necessary. Trees treated with tar for Pink Disease should be examined within a month, and if the fungus has spread, tarring should be tried again. If two applications of tar are found useless in checking the disease the affected parts should be cut out and burnt. On several estates where this mode of treatment has been adopted, Pink Disease has been reduced to a minimum. If tar is used to check Pink Disease it is essential that the work should be done under good supervision, for if done carelessly the money spent on it will be wasted. It is important also that diseased trees should be treated at an early stage. In certain cases, *e.g.* when the leaves of an affected branch have died, it is obviously hopeless to apply tar. The only thing to be done in such cases is to cut out and burn the diseased portions. The use of a concentrated Lime-Sulphur mixture has been tried instead of tar, but it is difficult to check the use of it and it is readily washed off by rain, hence it is not a good substitute and is not recommended.

Planters sometimes have difficulty in burning diseased branches on account of persistent rain. If it is impossible to burn the diseased parts directly, they should be drenched with a 10 per cent. solution of sulphate of copper, removed from the plantation and buried in the ground some distance away from the rubber trees. It must be remembered, however, that there is nothing so good as fire for the destruction of fungoid pests. In this connection mention may be made of the fact that another pink fungus, *Oospora sp.*, which is harmless, usually develops on wood in Malaya a few days after being burnt. This fungus has been several times mistaken for Pink Disease.

Where Pink Disease has appeared in an estate, a pest gang should be formed if not already established and the size of the gang should be such that it can go over the whole estate and treat diseased trees on the above lines once every three or four weeks. Pink Disease develops rapidly and any longer interval is too great to allow of it being dealt with effectively. Where a considerable amount of Pink Disease is present one can hardly expect to eradicate it completely so the expense

of maintaining a pest gang must be met. The rubber plantation industry is dependant on the health of the trees so it would be suicidal policy to grudge money for treating disease.

Any plants besides rubber which are affected by Pink Disease in the neighbourhood of estates should be destroyed as the fungus passes readily from one host to another. The manifestations of the disease on other plants are the same as on rubber.

If the measures indicated above are carried out, the disease should be kept under control, but any neglect of it will be dearly purchased. This disease of rubber trees is more common in Malaya at the present time than the die-back caused by *Diplodia* and it is a disease which will have to be watched carefully.

In conclusion we wish to express our thanks to Mr F. de la Mare Norris for kindly making the drawing from which Fig. 11 has been reproduced.

Summary.

1. The distribution, hosts, and mode of action of Pink Disease are described and its importance as a disease of plantation rubber is emphasised.

2. The various forms of *Corticium salmonicolor* are described. It is pointed out that the fungus is not a typical *Corticium* and that the pink incrustation is very frequently sterile.

3. *Corticium salmonicolor* often affects the wood as well as the bark of rubber trees. Its action on the wood is described in detail. The formation of tyloses appears to be a response to the presence of the fungus in the wood.

4. Pure cultures of *Corticium salmonicolor* have been established on salep agar and on *Hevea* wood.

5. Inoculation experiments both with natural material and with the fungus grown in pure culture have been successful.

6. *Treatment.* (*a*) Spraying is not recommended except in particular cases. (*b*) The disease is best dealt with either by cutting out infected branches, or by treating affected parts with tar. Detailed instructious are given as to the manner in which these operations should be carried out by a pest gang.

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